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ARTICLE

Occurrence of *Sturnira tildae* De La Torre, 1959 (Chiroptera: Phyllostomidae) in the state of Maranhão, Brazil

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Abstract. The bat genus *Sturnira* is widely distributed in the Neotropical region, from northwestern Mexico to northern Argentina, and four species occur in Brazil: *Sturnira lilium, Sturnira giannae, Sturnira magna,* and *Sturnira tildae.* The present study is the first to record *Sturnira tildae* in the state of Maranhão, Brazil, based on morphological and molecular diagnoses. The specimen was identified based on its cranial and morphometric traits. The diagnostic traits include discreetly bilobed inner upper incisors with a broad base, lower first and second molars with lingual cusps separated by shallow grooves, and forearm longer than 45 mm. The molecular sequences of Cytochrome C Oxidase Subunit 1 (COI) and 16S rRNA genes confirmed the morphological identification and thus the occurrence of *Sturnira tildae* in the Amazon biome of Maranhão. This record represents an eastward extension of the known distribution of the species in the Amazonia, to Cândido Mendes, Maranhão, within an area dominated by dense rainforest and influenced by tides.

Keywords. 16S rRNA; Amazon; COI; DNA barcode; Range extension; Stenodermatinae.

INTRODUCTION

Brazil has is the country with the third richest bat fauna in the world, with 181 species (including eight endemics) representing 68 genera and nine families (Garbino *et al.,* 2020; Velazco 2021). Much of this diversity is found in the Amazon biome, which is home to most of the species known to occur in Brazil (Bernard *et al.,* 2011). In this scenario, the Amazon of Maranhão, in the eastern extreme of the biome, is one of the Amazonian regions that have a fragmented distribution of bat species records, but which are gradually a number of recent studies have provided important insights into the distribution of chiropterans in this region (Bernard *et al.,* 2011; Oliveira *et al.,* 2011; Olímpio *et al.,* 2016; Lima *et al.,* 2018; Olímpio *et al.,* 2018; Mendes *et al.,* 2020).

Bats of the family Phyllostomidae are the most ecologically diverse group of mammals, and the second most speciose mammalian family, with 216 recognized species (Taylor & Tuttle, 2019). In recent years, a number of studies reporting the fauna of poorly-known areas associated with phylogenetic analyses, have advanced the scientific understanding of phyllostomid diversity, including the discovery of new species and the expansion of the inventories available for many genera, such as *Sturnira* (Miretzki *et al.,* 2002).

The phyllostomid genus *Sturnira* has a wide distribution in the Neotropical region, occurring from Mexico to northern Argentina (Gardner,

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2008). *Sturnira* is the most speciose phyllostomid genus, with 23 recognized species (Fleming *et al.,* 2020), of which, four occur in Brazil – *Sturnira lilium* (Geoffroy, 1810), *Sturnira giannae* Velazco & Patterson, 2019, *Sturnira magna* De La Torre, 1966 and *Sturnira tildae* De La Torre, 1959 (Garbino *et al.,* 2020). *Sturnira tilde* is found in Brazil, Colombia, Ecuador, the Guianas, Peru, Bolivia, Trinidad and Tobago, and Venezuela (Simmons, 2005; Gardner, 2008). In Brazil, this species has been recorded in the states of Amapá, Amazonas, Pará, Acre, Tocantins, Ceará, Pernambuco, Bahia, Mato Grosso, Goiás, Mato Grosso do Sul, Espirito Santo, Rio de Janeiro, São Paulo, Paraná, and Santa Catarina (Martins *et al.,* 2020).

Relatively few data are available on the diet or reproductive patterns of *S. tildae,* but this bat is assumed to be an important seed disperser, which contributes to the regeneration of forested areas (Simmons, 2005; Gardner, 2008). *S. tildae* shares a number of external characteristics with the other members of the genus, such as the lack of a tail, narrow and hairy interfemoral membrane, small ears, a small, broad nasal leaf, and posterior members and feet hairy, as far as the claws (Peracchi *et al.,* 2011). The color of the pelage varies from tones of yellow to brown, with some males having tufts of orangish or dark reddish-brown hair on the shoulders (Reis *et al.,* 2017).

The external measurements of *S. tildae* are similar to *S. lilium,* although *S. lilium* tends to be smaller (Gardner, 2008). Some studies point to the possibility of identification errors of identification related to the morphological similarities of these species and the overlap in their geographic distributions (Miretzki *et al.,* 2002; Sampaio *et al.,* 2016; Velazco & Patterson, 2017; Martins *et al.,* 2020). In areas that are in sympatry, *S. lilium* differs from *S. tildae* only by dental and morphometric characteristics (Simmons & Voss, 1988; Martins *et al.,* 2020). The present study uses morphological and molecular data to report on a new record of *S. tildae* from the state of Maranhão, which extends the known geographic distribution of the species in Brazil.

MATERIAL AND METHODS

Bats were collected in June 2016 in a fragment of forest located within the Amazon biome of the Brazilian state of Maranhão, in the municipality of Cândido Mendes. Specimen collection followed the procotol of Pacheco (2004) and permits were duly provided by the Brazilian federal agency for the environment (IBAMA/SISBIO permit number 42670-3). The vegetation of the study region is dense rainforest typical of the Amazon biome, and it has a humid equatorial climate, as well as being influenced by the tides of the Maracaçumé River, which crosses the town of Cândido Mendes (Bandeira, 2013).

The specimen described here was collected using a 3 m high and 12 m long mist-net, with a 25 mm mesh. The age of the specimen was determined in the field based on the ossification of the phalangeal epiphyses, and its sex and reproductive status were also recorded (Brunet & Austad, 2004). The specimen was photographed, euthanized, labeled, and stored on ice for transportation to the Genetics and Molecular Biology Laboratory (GENBIMOL) of Universidade Estadual do Maranhão (UEMA) in Caxias, Maranhão, where it was weighed and measured, and a sample of a muscle tissue was extracted, which was stored in ethanol 70° for the molecular analyses.

The skull was extracted through the buccal aperture. Once clean, the skull was clarified with 10% peroxide and dried in a stove at 30°C, after which, it was labeled and stored in a clean recipient. The specimen was fixed in formaldehyde and preserved in ethanol at UEMA's Laboratory of Genetics and Molecular Biology, on the campus of Caxias, and then transferred to the mammal collection of the Federal University of Paraíba, in João Pessoa, Brazil, where is it deposited. The specimen was identified based on its external traits and craniometric measurements, following Vizzoto & Taddei (1973), Simmons & Voss (1998), and Reis *et al.* (2013, 2017). Measurements taken include the length of right and left forearms, ear, tragus, foot, greatest length of the skull, basal length and condylobasal length, width of the brain case and the mastoid, zygomatic width, the length of the upper tooth row, and the width across the molars (Table 1). Body mass, after the specimen had been dead for hours, was determined using a precision balance after euthanasia of the specimen.

The total DNA was extracted from the muscle tissue using Promega's Wizard Genomic DNA Purification kit, following the default protocol. Two mitochondrial genes, Cytochrome C Oxidase Subunit 1 (COI) and rRNA 16S, were amplified by Polymerase Chain Reaction (PCR) using the primers LCO-1490 and HCO-2198 for the COI gene (Folmer *et al.,* 1994) and L1987 and H2609 for the rRNA 16S (Palumbi *et al.,* 2002). The samples were sequenced by the dideoxyterminal method of Sanger *et al.* (1977) in an ABI Prism™ 3500 (Applied Biosystems, USA) automatic DNA sequencer, using the Big Dye kit. The sequences were edited and aligned in BIOEDIT 7.0 (Hall, 1999), with the genetic divergence matrices being complied in MEGA X (Kumar *et al.,* 2018) using the Kimura 2-parameter algorithm. The sequences were plotted in the BOLD Systems v4 (www.boldsystems.org) and BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi) platforms to confirm their identification and determine their degree of similarity with sequences of other *Sturnira* species.

RESULTS

We collected an adult female *S. tildae* (field number: RRM 117, voucher: UFPB 11757) in June 2016 from a farm located within the urban perimeter of the town of Cândido Mendes (01°27′21″S, 45°43′32″W), in the Amazon biome, within an area containing remnants of rainforest. The length of the right forearm was 45.77 mm, while its left forearm was 46.34 mm. The length of ear of the specimen was 12.95 mm, the tragus 5.46 mm, and the foot 12.45 mm. The specimen was a non-lactating

Table 1. Craniometric measurements (in mm) of the *Sturnira tildae* specimen from Cândido Mendes, Maranhão, Brazil (present study), together with the values recorded from specimens of the same species from Recife (Pernambuco), Morretes (Paraná), and French Guiana.

Measurements (mm)	Present study	Martins *et al.* (2020)	Miretzki *et al.* (2002)	Simmons & Voss (1998)	
	Cândido Mendes	Recife	Morretes	French Guiana	
	Female (n=1)	Female (n=1)	Male (n=1)	Female (n=13)	Male (n=11)
Length of the skull	23.6	23.6	—	—	—
Basal length	18	—	18.5	—	—
Condylobasal length	21.6	22.6	21.71	21.26	21.65
Length of upper tooth row	7.1	7.6	7.10	6.87	6.81
Postorbital width	6.2	6.5	6.55	6.16	6.21
Width of the mastoid	11.9	—	12.92	12.76	12.96
Zygomatic width	14		14.80	14.15	14.37
Width of the braincase	10.1	10.2	10.96	10.80	10.94
Width across the molars	8	7.9	—	8.21	8.02

which had ectoparasites, orange-brown pelage, with the venter paler than the dorsum, and dark hairs around the eyes (Fig. 1).

Sturnira tildae was also identified based on the following set of morphological cranial traits: small brain case and broad, high rostrum, slightly bilobed upper incisors with a wide base and narrower points, and first and second lower molars with lingual cusps separated by shallow grooves with no vertical border (Fig. 2A-D and Table 1).

The present study provides the first record of *S. tildae* from the Brazilian state of Maranhão, and only the fifth for the Brazilian Northeast geographic region (two from Bahia state – Faria & Baumgarten, 2007; Faria, 2006, one from Pernambuco; Martins *et al.,* 2020 and one from Ceará – Novaes & Laurindo, 2014). The geographically closest locality to Cândido Mendes with records of *S. tildae* is Santa Barbara, in the neighboring state of Pará, which is *ca.* 280 km due west (Fonseca, 2006). There is also a record from the municipality of Muaná, in Pará (Marques-Aguiar *et al.,* 2002), which is 402 km west of Cândido Mendes. Both these localities are within the Amazon biome. South of Maranhão, the nearest locality is in the municipality of Goiatins, in Tocantins state (Maas *et al.,* 2018), which is 724 km to the south, in the Cerrado savanna biome, while the nearest record to the east is from the municipality of Barbalha, in Ceará state, at a distance of 967 km, in the Caatinga biome (Novaes & Laurindo, 2014) (Fig. 3). The present study extends the known distribution of *S. tildae* to the eastern extreme of the Amazon biome.

Figure 1. *Sturnira tildae* (female, RRM 117) collected em Cândido Mendes, in the Amazon biome of Maranhão state, Brazil.

The sequence of the rRNA 16S gene obtained from the specimen analyzed in the present study diverged by 0.4% from those of *S. tildae* from French Guiana and by 4.8% from *S. lilium*. The BLAST search identified a genetic similarity of 100% with *S. tildae* from French Guiana. In the case of the COI gene sequences, intraspecific genetic divergence between the study specimen and sequences from French Guiana, Surinam, Ecuador, and Guyana was 0.92-1.58%, while it was 11.8% in relation to *S. lilium*. In the BOLD Systems platform, the sequence returned a similarity of 99.09% with *S. tildae* from French Guiana (Table 2). These results are consistent with the morphological and craniometric identification of the specimen, and confirm the occurrence of *S. tildae* in the Amazon biome of Maranhão state.

DISCUSSION

In the present study, the cranial and morphological measurements of the specimen, are typical of *S. tildae,* as defined by De La Torre (1959), Simmons & Voss (1998), Miretzki *et al.* (2002), López-Baucells *et al.* (2016), Velazco & Patterson (2019), and Martins *et al.* (2020). The cranial and dental measurements of the specimen (skull, basal, and condylobasal lengths, the length of the upper tooth row, and the postorbital and zygomatic widths, the width of the braincase, and the width across the molars) are all very similar to those of the *S. tildae* specimens collected in Recife, Pernambuco (Martins *et al.,* 2020) and Morretes, Paraná (Miretzki *et al.,* 2002). This indicates that differences due to the geographic or sexual variation are negligible.

The species *S. tildae* has great morphological similarities with *S. lilium,* making identification difficult. (Simmons & Voss, 1998). A number of diagnostic traits can nevertheless be used to distinguished the two taxa, including the length of the forearm, which exceeds 45 mm in *S. tildae,* but never exceeds this value in *S. lilium* (Simmons & Voss, 1998; Reis *et al.,* 2013, 2017; Martins

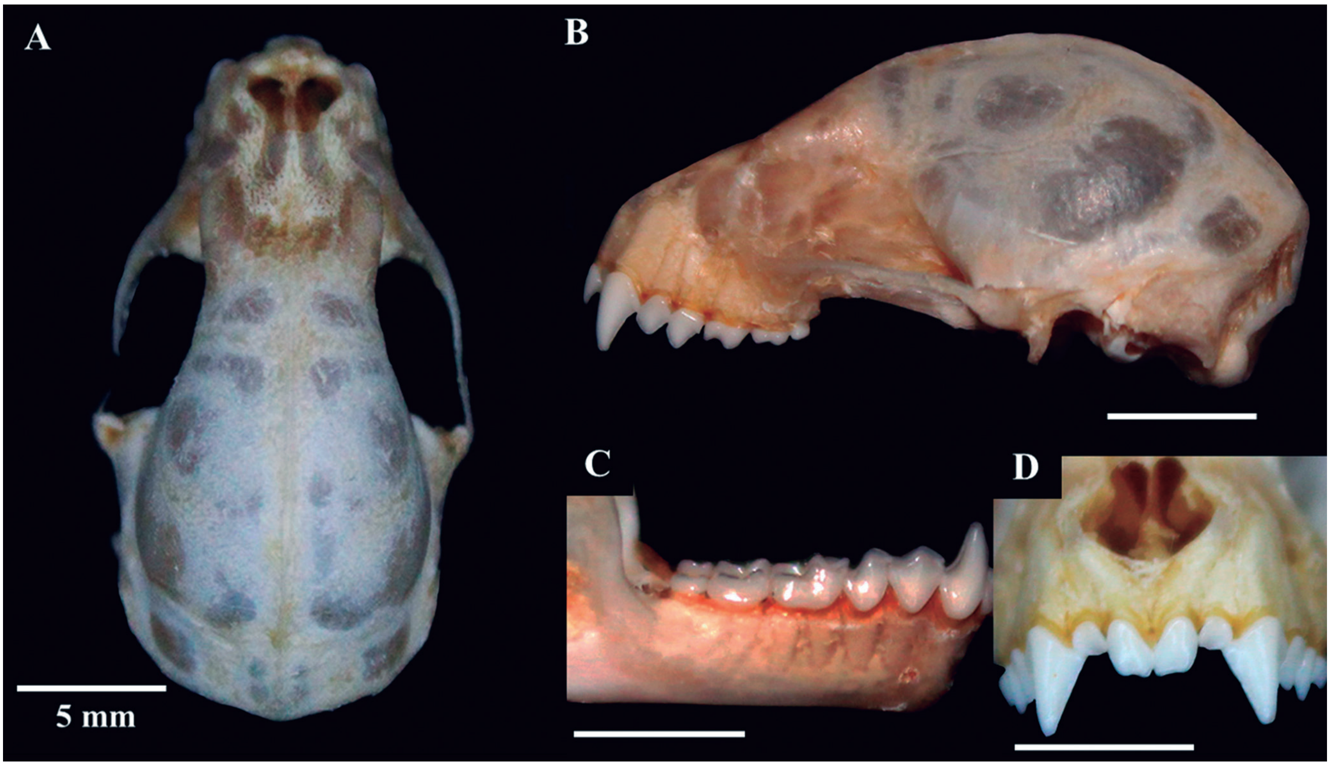

Figure 2. Skull of *Sturnira tildae* (RRM 117). (A) dorsal view showing the small braincase; (B) lateral view showing the broad, elongated rostrum; (C) lateral view of the mandible showing the first and second molars with lingual cusps separated by shallow grooves; (D) frontal view of the upper incisors, showing the slightly bilobed internal incisors with a wide base. Scale bar: 5 mm. Source: Olímpio, A.P.M.; Lima, A.C.S.

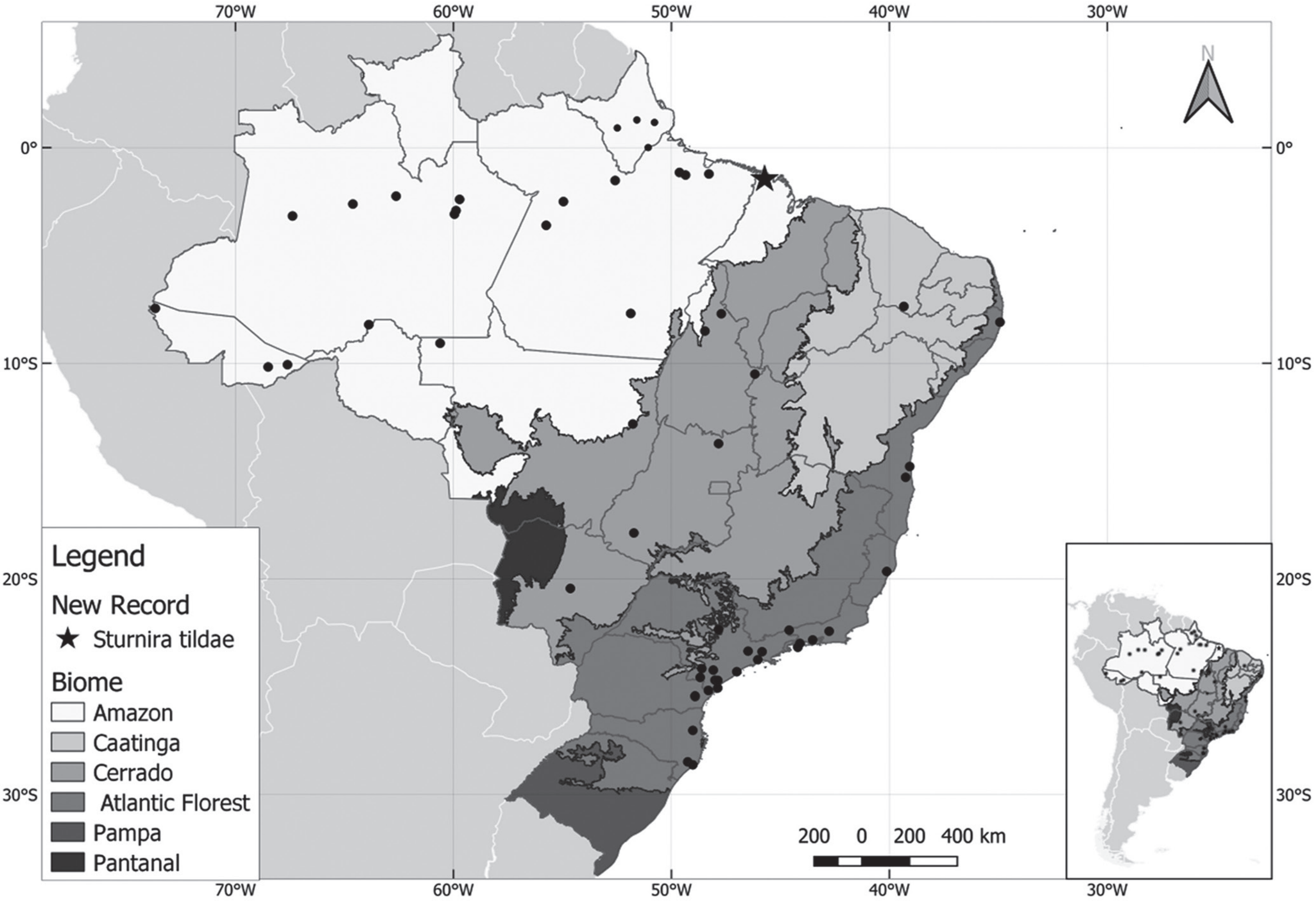

Figure 3. Geographic distribution of *Sturnira tildae* in Brazil. The locality reported in the present study in the state of Maranhão is shown by a star. The Brazilian biomes are shaded in black (Pantanal), darker to lighter gray (Pampas, Atlantic Forest, Cerrado, and Caatinga, in this order), and white (Amazon). Source: adapted from Martins *et al.* (2020).

Table 2. Interspecific genetic divergence in *Sturnira tildae* based on the sequences of the COI gene, using the 2-parameter Kimura algorithm. *Lophostoma silvicola* and *Carollia perspicillata* were included as outgroups. Legend: GUY = Guyana, SUR = Suriname, ECU = Ecuador, FGU = French Guiana, CM = Candido Mendes, CX = Caxias (Maranhão).

Species/specimens	**Genetic Divergence (%)**											
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
1. *S. tildae* JF455913 GUY	—											
2. *S. tildae* JF455917 GUY	0.17											
3. *S. tildae* JQ601103 SUR	0.34	0.52										
4. *S. tildae* JF447740 SUR	0.52	0.69	0.52									
5. *S. tildae* JF449229 ECU	0.52	0.69	0.87	0.69								
6. *S. tildae* JF449230 ECU	0.34	0.52	0.69	0.52	0.17							
7. *S. tildae* KU295485 GUF	0.00	0.18	0.36	0.55	0.55	0.36						
8. *S. tildae* HG003314 GUF	0.17	0.34	0.52	0.69	0.69	0.52	0.18					
9. *S. tildae* RRM117 CM	1.04	1.22	1.40	1.58	1.58	1.40	0.92	1.22				
10. *S. lilium* CESC52 CX	11.5	11.6	11.5	11.7	12.2	11.5	12.2	12.2	11.8			
11. *L. silvicola* CESC51CX	23.5	23.8	23.5	22.8	23.1	23.5	23.8	23.8	24.4	22.7		
12. *C. perspicillata* CESC9CX	27.0	26.6	27.4	27.4	26.3	26.6	28.2	27.4	27.6	24.5	24.7	—

et al., 2020); the lighter coloration of the pelage of the venter in *S. tildae* in comparison with *S. lilium,* and the larger lengths of the ear, tibia, wing, and condylobasal length in *S. tildae* (Fazzolari-Correa, 1995; Davis, 1980). All these differences were also observed in the *S. tildae* specimen analyzed in the present study.

The dentition provides the most reliable evidence for the differentiation of the two species, given the presence of bilobed internal superior incisives and wider in *S. tildae,* than observed in *S. lilium* (Eisenberg, 1989), as well as the lingual cusps of the first and second molars of the mandible, *S. lilium* has high lingual cusps, separated by a deep vertical notch between the first and second molars and in *S. tildae* these cusps are low and separated by shallow notches (Miretzki *et al.,* 2002). These authors considered the differences in the configuration of the cusps of the mandibular molars to be completely reliable for the distinction of the specimens of *S. lilium* and *S. tildae* from Paracou, in French Guiana, and this conclusion was further confirmed in the present study.

The analyses of the 16S rRNA and COI genes revealed a high degree of similarity, in both cases, between the specimen presented here and *S. tildae* from French Guiana, with a genetic divergence of less than 2% in comparison with the specimens from of different localities, which is consistent with the 2% intraspecific threshold of the COI marker established for bats by Clare *et al.* (2011). Despite the fact that *S. lilium* and *S. tildae* present major morphological similarities, the analysis of the rRNA 16S sequences indicated a divergence of 4.8% between the *S. tildae* specimen presented here and *S. lilium,* increasing to 11.8% for the COI gene. The genetic data thus support conclusively the morphological and craniometric analyses, and confirm the occurrence of *S. tildae* in Maranhão.

As for distribution, *S. tildae* is considered a common species within its geographic distribution (Wilson 1996; Miretzki *et al.,* 2002), with a distinct preference for more humid forest environments (Trajano, 1984; Marques, 1985; Brosset *et al.,* 1996; Simmons & Voss, 1998; Miretzki *et al.,* 2002; Novaes & Laurindo, 2014), such as the locality surveyed during the present study. Even so, *S. tildae* has been captured relatively infrequently in most bat field studies, in all different biomes (Oliveira, 2000; Marques-Aguiar *et al.,* 2002; Miretzki *et al.,* 2002; Carvalho *et al.,* 2013; Luz *et al.,* 2013; Menezes *et al.,* 2015; Maas *et al.,* 2018; Cláudio *et al.,* 2020; Martins *et al.,* 2020).

In Brazil, *S. tildae* has been recorded in the Amazon, Atlantic Forest, Cerrado, and Caatinga biomes. In the Amazon biome (IBGE, 2019), there are records from the Brazilian states of Amazonas, Acre, Rondônia, Pará, Amapá, Roraima, and Mato Grosso (Tavares *et al.,* 2008; Reis *et al.,* 2013; Novaes & Laurindo, 2014; Reis *et al.,* 2017), and the closest locality to the present study site is 280 km west, in Pará. This is the fifth record of the occurrence of *S. tildae* in the Brazilian Northeast. The previous records included one from the Caatinga biome, in Barbalha, Ceará state (Novaes & Laurindo, 2014), and three from the Atlantic Forest, in Ilhéus (Faria & Baumgarten, 2007) and Una (Faria, 2006), both in Bahia, and Recife in Pernambuco state (Martins *et al.,* 2020). Barbalha is very close – about 20 km – to Floresta Nacional do Araripe. The area is considered to be a relict of Atlantic Forest in the middle of the Caatinga (Silveira *et al.,* 2019).

CONCLUSIONS

The present study recorded the occurrence of *S. tildae* in the Amazon biome of the Brazilian state of Maranhão, based on both morphological and molecular evidence. This is the first record of the species for the state, and the fifth for the Brazilian Northeast, and extends the known distribution of the species to the municipality of Cândido Mendes, 280 km east of the nearest locality in the Amazon biome. The findings of the study reinforce the need for attention when identifying the species of the genus *Sturnira,* in particular in areas where *S. lilium* and *S. tildae* are likely to be captured together and then released, given that the considerable morphological similarities of these sympatric taxa may result in errors of identification. As *S. tildae* occurs more frequently in moist forests, new records are expected in other Amazonian areas in Maranhão, as well as in Brejos de Altitude habitats within the Caatinga biome (see Tabarelli & Santos, 2004).

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AUTHORS' CONTRIBUTIONS

ACSL: Conceptualization, Writing – original draft, Visualization, Investigation, Data curation, Formal Analysis, Validation. **CLSC:** Conceptualization, Visualization, Writing – review & editing. **SBM:** Visualization, Methodology, Data curation, Writing – review & editing. **FHSC:** Visualization, Methodology, Writing – review & editing. **BATPC:** Supervision, Data curation, Writing – review & editing. **ECF:** Supervision, Writing – review & editing, Funding acquisition, Project administration, Validation. **MCB:** Supervision, Writing – review & editing, Formal Analysis, Validation, Data curation, Funding acquisition, Project administration. All the authors actively participated in the discussion of the results, they reviewed and approved the final version of the paper.

CONFLICT OF INTEREST

Authors declare that there is no conflict of interest.

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